SOME BIOLOGICAL AND ECOLOGICAL OBSERVATIONS ON *SPHAEROPLEA ANNULINA* (ROTH) AG. (CHLOROPHYCEAE) IN NORWAY

Anders LANGANGEN
Hallagerbakken 82b, 1256 Oslo, Norway

ABSTRACT - *Sphaeroplea annulina* (Roth.) Ag. has been found in three small rainwater pools near the sea in southern Norway. Observations on the growth and developement of the alga were made in 1992 and 1993. In general this alga develop very quickly and complete its life-cycle in 3-4 weeks in its natural environment. These figures are confirmed in culture experiments. The alga has been found growing under optimal conditions in fresh water and slightly brackish water (0,02 - 1,3 g l^{-1} salt). These observations made both in nature and under laboratory experiments. Due to evaporation of the water in these pools the alga must stand a higher variation of salt content. In nature I observed wellgrowing specimens in salt content to 2,12 g l^{-1}. In the culture experiments the oospores germinated in water of 3,6 g l^{-1} salt content. The germination in this type of water was slower and the development of the alga was prolonged. In the experiment with filaments they survived only in water with a salt content below 2,7 g l^{-1}. The Norwegian specimens fit the general description of the alga, but the variation in the two characters width of the cells and diameter of the oospores was less than in material procured elsewhere in Europe. *Sphaeroplea annulina* is also found in eutrophic lakes, but the special adaptations like fast life-cycle and massproduction of oospores support the idea that this alga has specialized to survive in temporary waters, where there is little competition from other plants.

RÉSUMÉ - *Sphaeroplea annulina* (Roth) Ag. a été trouvé dans trois petites flaques d'eau de pluie à proximité de la mer dans le sud de la Norvège. Des observations sur la croissance et le développement de cette algue ont été réalisées en 1992 et 1993. Elle se développe en général très rapidement et accomplit son cycle de vie en 3 à 4 semaines dans son environnement naturel, ce qui a été confirmé dans des cultures expérimentales. Elle trouve les conditions optimales de sa croissance dans l'eau douce ou faiblement saumâtre (0,02-1,3 g l^{-1} de sel). Ces observations ont été réalisées à la fois dans la nature et en expérimentation en laboratoire. L'évaporation de l'eau de ces flaques provoque une concentration plus élevée de la salinité à laquelle l'algue doit s'adapter. Dans la nature j'ai observé des spécimens en croissance active dans des eaux contenant 2,12 g l^{-1} de sel. En culture des oospores ont germé dans de l'eau salée à 3,6 g l^{-1}; la germination dans ce type d'eau était plus lente et le développement de l'algue était prolongé; les filaments eux ne survivaient que dans de l'eau avec une concentration en sel inférieure à 2,7 g l^{-1}. Les spécimens de Norvège sont conformes à la description générale de cette espèce, mais la variation de deux caractères, longueur des cellules et diamètre des oospores, était plus faible que dans le matériel provenant d'autres régions d'Europe. *Sphaeroplea annulina* se rencontre aussi dans des lacs eutrophiques mais certaines particularités, comme un cycle de vie rapide et une production en masse d'oospores, permettent de supposer que cette algue est adaptée à la survie dans des eaux temporaires, là où il y a peu de compétition avec d'autres plantes (traduit par la rédaction).

KEY WORDS : Chlorophyta, *Sphaeroplea annulina*, ecology, Norway.

INTRODUCTION

In 1991 I found *Sphaeroplea annulina* in Langesund in South-Norway (Langangen, 1992). Both in 1992 and 1993 I visited the localities several times.

Ecological and biological observations on *Sphaeroplea* in Norway are found in Langangen (1992). General information on biology and especially reproduction are found in many classical works on this alga (Fresenius, 1851; Cohn, 1856; Heinricher, 1883; Rauwenhoff, 1888; Klebahn, 1899; Fritsch, 1929 and Palik, 1950).

Sphaeroplea annulina is found in fresh water and slightly brackish water (Skuja, 1927; Palik, 1950; Christensen, 1954, 1971). The alga also appear frequently in fountains and springs in different botanical gardens throughout Europe (Fritsch, 1906; Fritsch, 1929; Palik, 1950). Some ecological information on the species are given by Lowe (1924) and by Cambra & Couté (1988).

DESCRIPTION OF THE LOCALITIES

The localities of *Sphaeroplea* can be found in Krogshavn situated in the small coastal town of Langesund in southern Norway. Krogshavn was earlier a brackish water area which now has been filled out and redeveloped into a local recreation ground. Here I found *Sphaeroplea* for the first time on 06.07.1991 (Langangen, 1992).

The bedrock here is cambro-silurian limestone with small rainwater pools scattered all over the area. These pools are most often found in small hollows in the limestone, probably of glacial age. All the pools are in varying degrees influenced by the nearby sea. The content of salt in the pools will therefore vary from brackish to fresh water. In summer most of the pools normally contain fresh water from rainfall, if they are not dried out. In the autumn the content of salt is higher, as a result of the storms normally taking place at this time of the year. In this period the pools near the sea often contain marine algae thrown up from the sea.

On 27.07.92 I found *Sphaeroplea annulina* in three small rainwater pools located along a line 5-30 meters from the sea and 0,5-1,0 meters above sea level.

Loc. 1 The locality from 1991. 2-3 m long, 0,5-1,0 m broad and 20-25 cm deep.

Loc. 2 Closer to the sea. 2-3 m long, 0,5 m broad and 20 cm deep. At a lower water level this pool divides in two smaller pools.

Loc. 3 Close to the sea in an area 1-2 m long, 0,5 m broad and 15-20 cm deep.

The localities are shown in figure 1.

MATERIAL AND METHODS

During the field studies water samples were taken in bottles for water analyses. pH, conductivity and salt content were measured at the spot. Calcium and chloride were measured later. pH was measured with a Hellige Comparator; conductivity and salt content with a Hach Conductivitymeter Model 44600/CND/TDS Meter. Chloride

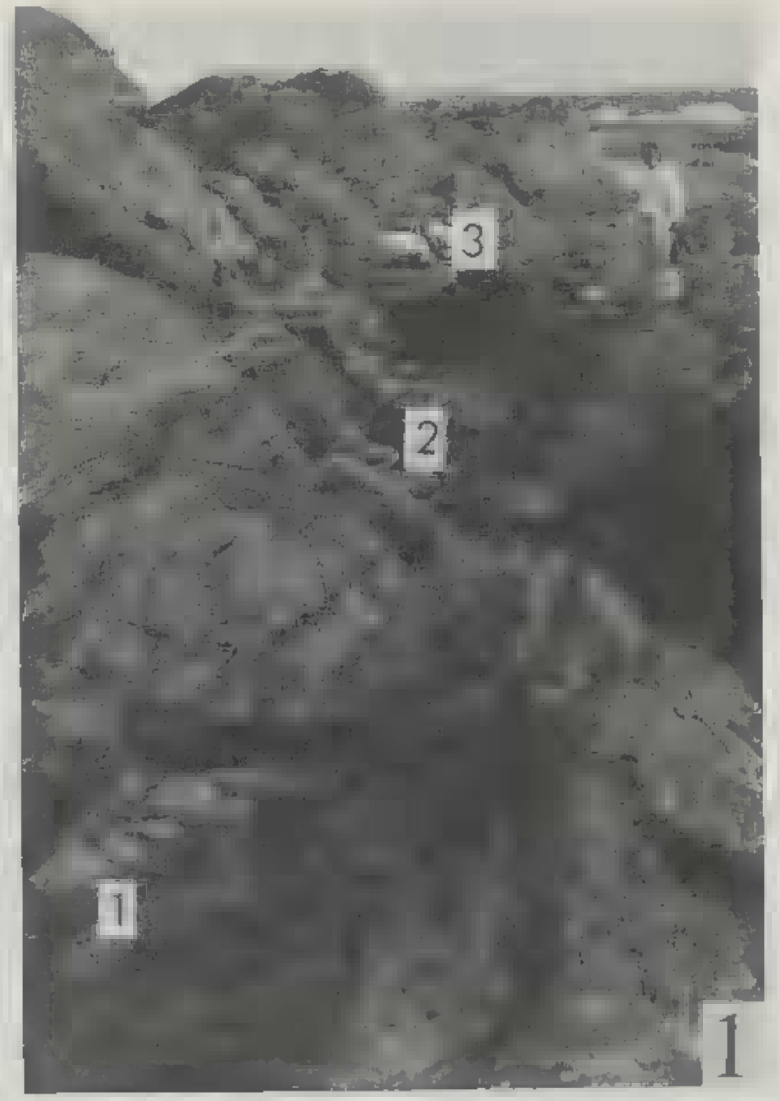
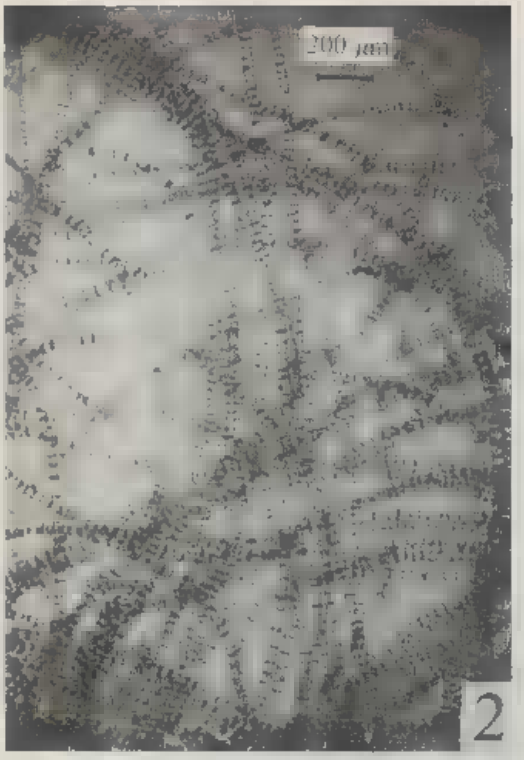


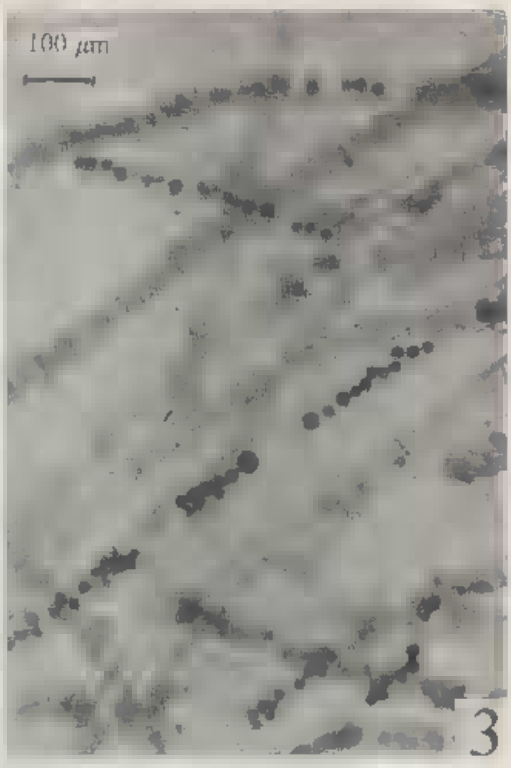
Figure 1. The localities with *Sphaeroplea annulina* by Langes und.The three localities are marked by a number. 1. is loc. 1, in the left corner with green tufts of the alga. 2. is loc. 2, in the middle of the photo, now divided in two parts. 3. is loc. 3, hidden behind the white stone. *Photo 29.07.1992.*

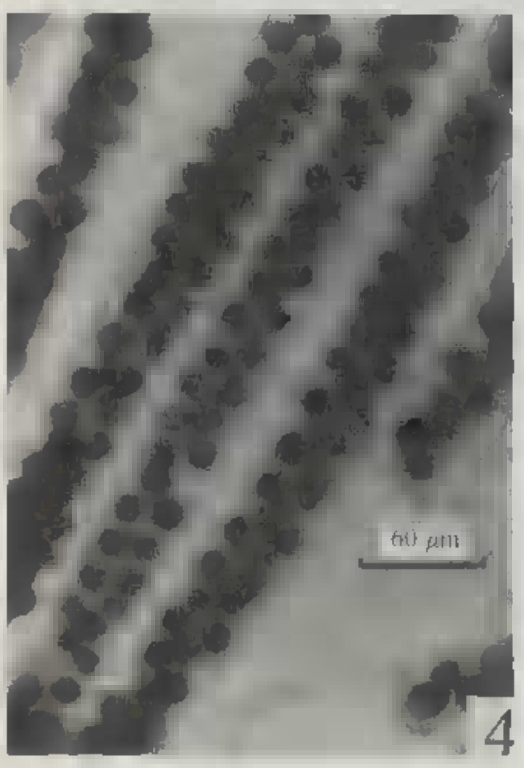
and calcium were measured with Aquamerck 11106 Chlorid and Calcium with Aquamerck 11110 Calcium.

The growth experiments took place in the spring and summer of 1993. The main purpose of the experiments was to find out how this alga reacted to different contents of salt in the water. In order to obtain these differences in salt content I used water from the localities and mixed this with salt (NaCl) or distilled water.

Specimens of the alga were taken from the localities, and grown in small culture-glasses of 500 cm^3. These were placed in a window facing north to northeast without any extra source of light. Here they were exposed to sun early in the morning and late in the evening. Observations on the growth and development were made







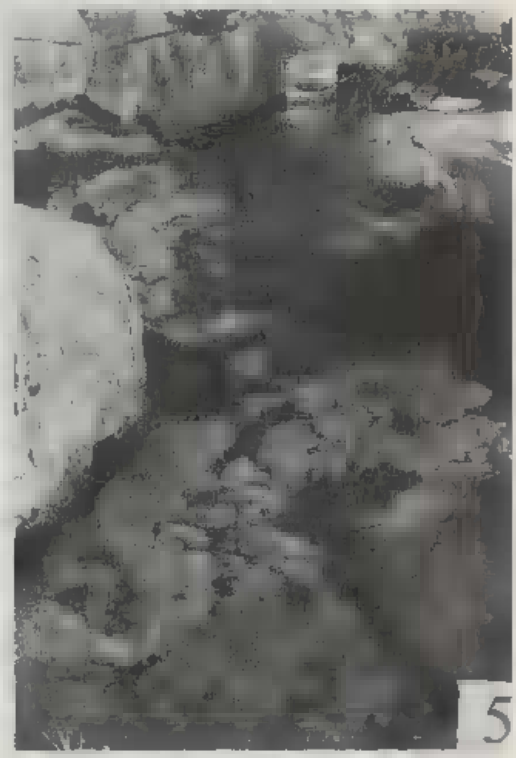


Figure 2. *Sphaeroplea annulina.* Vegetative filaments. From loc.1. *Photo 27.07.1992.* - Figure 3. *Sphaeroplea annulina.* Vegetative filaments with cells tranformed to oogonia. The oogonia ▬ filled with ova. From loc. 2. *Photo 27.07.1992.* - Figure 4. *Sphaeroplea annulina.* Oogonia filled with red oospores. Loc. 1. *Photo 04.08.1992.* - Figure 5. Part of loc. 3. Green masses of *Sphaeroplea annulina. Photo 29.07.1992*

regularly. All normal developed filaments of *Sphaeroplea annulina* were brought back to the original pool after the completion of the experiment.

RESULTS

a. Field observations in 1992

When I visited the area in early July 1992, most of the pools were dried out as a result of the extremely dry and warm weather in May and June that year. During the first weeks of July rain filled up the pools, but later they dried out again. From the middle of July the rainfall was sufficiently high to give persistent water content in the pools. Most probably this happened around 18-21 July when the precipitation was 24,5 mm according to measurements made at Porsgrunn Brannstasjon (Firestation), situated 10 km north of the localities.

When I visited Krogshavn on 26.07.92 the locality studied in 1991 contained *Sphaeroplea*. Later the water level varied a lot. A rainfall the next day (27.07 / 11 mm) filled the pools with water, but two days later the water level was low again (because of the outflow and evaporation). The observed variations in chemical parameters was due to the differences between evaporation and rainfall.

The development from oospores (c. 20 July) to mature status took about three weeks. The results of my observations are given in table I.

b. Field observations in 1993

The situation in 1993 was more complicated, since the weather was more fluctuating. This year the growth of *Sphaeroplea annulina* presumably started during the last weeks of June. When I visited the localities on 05.07.93, the pools had already or had nearly dried out. After a rainfall 10.07.93 the alga started again, in loc. 1 and loc. 2 some filaments must have survived, but in loc. 3 the old oospores germinated to form new filaments. This year I did not observe any red filaments in the pools.

The results of my observations are given in table II.

c. Growth experiments.

These experiments are qualitative only, and gives no answer on the nutritional demands of the alga.

1. Growth of oospores

The experiment started on 30.05.1993 and ended on 29.06.93. I used bottom sediments and water from loc. no. 2.

The salt content in the 13 culture glasses varied between 0,05 g l^{-1} - 6,15 g l^{-1} (CND 107 µS cm^{-1} - 12300 µS cm^{-1}).

Only oospores in water with a salt content below 3,6 g l^{-1} germinated. In distilled water oospores did not germinate. It seems that a high salt content prolonged the germination and the development of the alga. In these environments the alga was also poorly developed, and contained fewer oospores.

Table I. Some physical/chemical parameters and biological status for loc. 1-3 in 1992.

Loc. 1

Date	Rain	CND µS cm^{-1}	Salt g l^{-1}	pH	Ca mg l^{-1}	Cl mg l^{-1}	t^{0} C
27.07.92	11mm	350	0,18	8,8	6,0	47,5	20,7
29.07.92	-	470	0,24	8,8	12,0	45,0	22,6
31.07.92	-	470	0,24	8,8	10,0	60,0	21,2
04.08.92	7,7mm	447	0,22	>8,8	6,0	75,0	-
10.08.92	10,5mm	447	0,22	8,0	6,0	70,0	-

Date	Short description of the biological conditions
27.07.92	Vegetative filaments only. Fig. 2.
29.07.92	Vegetative filaments. In many filaments the cells have been changed and [illegible] acting as oogonia or antheridia. Fig. 3. The sexual process could now be followed in detail. The water had a brown colour. Fertile *Oedogonium* is mixed with *Sphaeroplea*.
31.07.92	The quantity of the alga in the small pool has increased considerably. The filaments contain mostly cells with ova or empty antheridia. The number of vegetative filaments are low. Spermatozoids swim around in the water and inside the oogonia. Some ova have been fertilized and have changed to green, verrucose oospores. But most of the contents in oogonia are still round, fertilized (most probably) ova.
04.08.92	The alga are now reddish in contrast to earlier green.This is due to the change in colour of the oospores, which now are reddish. Many cells are now filled up with oospores. Vegetative filaments [illegible] not found any longer. Threads with empty cells or antheridia [illegible] common. Filaments 30-45 µm broad. Oospores 15-20 µm in diameter in two rows. Fertile *Oedogonium*.
10.08.92	The alga in the pool are now red. The filaments contained only ripe, red oospores which filled up the cells (Fig. 4) and many empty antheridia.
05.09.92	There was no visible filaments of *Sphaeroplea* in the water now. In the bottom sediment there were masses of red oospores.

Loc. 2

Date	CND µS cm^{-1}	Salt g l^{-1}	pH	Ca mg l^{-1}	Cl mg l^{-1}	t^{0} C
27.07.92	180	0,09	8,4	3,0	17,5	19,5
29.07.92	400	0,20	8,8	8,0	25,0	24,0
31.07.92	460	0,23	8,8	8,0	30,0	20,8

Date	Short description of the biological conditions
27.07.92	Few vegetative filaments. Most cells have been converted to oogonia or antheridia. Fig. 3.
29.07.92	Almost only filaments with oogonia with ova or empty antheridia. Only few vegetative threads.
31.07.92	Only oospores and empty antheridia. The oospores are green, verrucose, and they fill up the cells. Cell diameter is in average 45 µm. A few vegetative cells [illegible] still found. The locality have nearly dried out.

Loc. 3

Date	CND µS cm^{-1}	Salt g l^{-1}	pH	Ca mg l^{-1}	Cl mg l^{-1}	t^{0} C
27.07.92	280	0,14	7,6	2,0	27,5	18,5
29.07.92	450	0,23	8,8	8,0	40,0	20,7
31.07.92	580	0,29	8,8	8,0	55,0	19,8

Date	Short description of the biological conditions
27.07.92	The filaments have cells with ova or empty antheridia. Big quantities of spermatozoids are swimming around in the water.
29.07.92	Filaments with oogonia and antheridia are dominating. Only a few vegetative threads are found at this point Fig. 5.
31.07.92	Still few vegetative threads. Cells with oospores and empty antheridia dominate. The oogonial cells contain a mixture of round (fertilized ova) and verrucose oospores. The colour of these oospores are green or yellowgreen.

Germination with optimal growth of the alga was obtained in the concentrations of salt 0,05 g l^{-1} - 0,20 g l^{-1}.

In the optimal cultures the steps in the development of the alga were as follows:

1. From oospore to vegetative filaments ♦ 10 days
2. The commencement of differentiation in vegetative filaments to green oospores ♦ 13 days
3. From green oospores to ripe, red oospores ■ 7 days

The total number of days needed to complete one cycle was here c. 30 days. In the optimal salt area the alga was well developed. The values for some important characters were:

1. Width of cells 20 - 37,5 µm
2. Oospore diameter 17 - 20 µm
3. Number of oospores in each cell 1 - 54

In each cell the oospores were arranged in 1- 2 rows.

2. Growth of filaments

The experiment started on 06.07.1993 and ended on 27.07.93. Material used contained filaments with vegetative cells and cells with masses of ova (from loc. 1).

The salt content in the 21 culture glasses varied between 0,02 g l^{-1} -3,84 g l^{-1} (CND 50 µS cm^{-1} - 7680 µS cm^{-1}).

Only filaments in water below salt content 2,7 g l^{-1} survived. The alga did not survive in distilled water .

Optimal growth of the alga was in the salt area 0,02 g l^{-1} - 1,30 g l^{-1}. In higher salt concentrations only a small part of the cells content ripened to become red oospores. In water with a 0,5 g l^{-1} salt content only some filaments developed into ripe, red oospores. These filaments were narrow and filled only one row of red oospores (Fig. 6). In the optimal area (0,3 g l^{-1}) there were many cells filled with red oospores in double rows (Fig. 7).

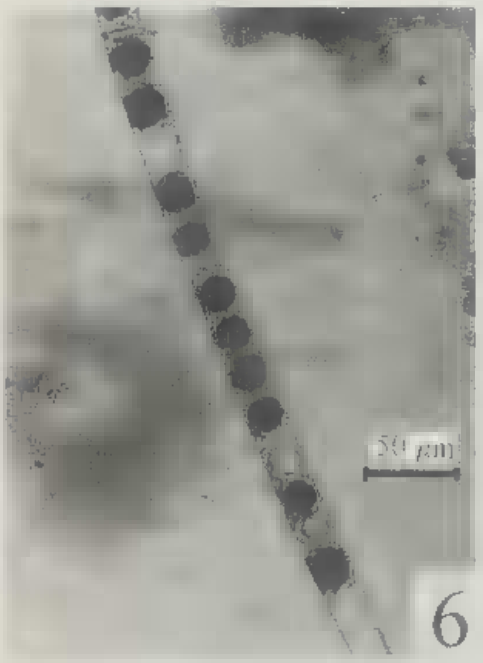
In the optimal cultures the steps in the development of the alga were:

1. From cells with ova to green oospores ♦ 2-6 days
2. From green oospores to red oospores ■ 8-9 days

In the optimal salt area the alga developed well. The values for some important characters are:

1. Width of cells 15 - 40 µm
2. Oospore diameter 20 - 25 µm
3. Number of oospores in each cell 6 - 75

In each cell the oospores develop in 1 - 2 rows.



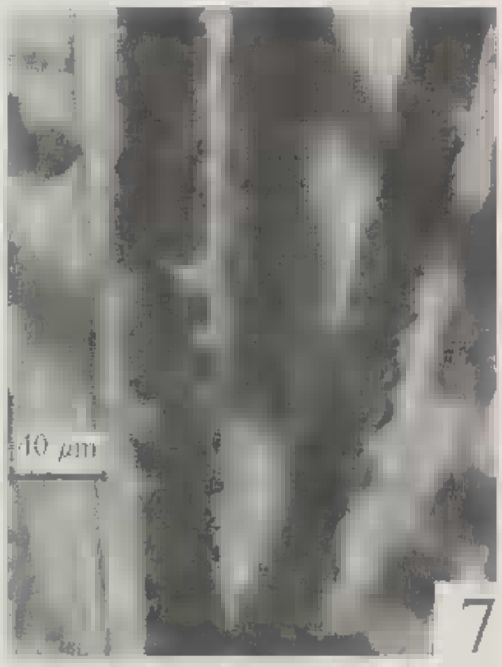


Figure 6. *Sphaeroplea annulina*. From experiment no. 2., water with 0,5 g l^{-1} salt. In cells with red oospores in one row. *Photo 13.08.93.* - Figure 7. *Sphaeroplea annulina*. From experiment no. 2. Salt content 0,27 g l^{-1}. The cells are filled up with red oospores in 1-2 rows. *Photo 24.07.93.*

DISCUSSION

Sphaeroplea annulina is found on all continents, except Australia (Gauthier-Lièvre, 1941). In Europe the species is found scattered, and it occurs only rarely (Palik, 1950). The known Scandinavian distribution is given in Langangen (1992). In addition there are three old finds in Sweden, two in Uppsala in 1877, and one in Jönköping (see exsiccatae).

Sphaeroplea is found in different kind of waters, but often reported from temporary pools near the sea. It is also found in eutrophic lakes (Christensen, 1971): i.e. one high mountain lake in India (Randhawa, 1958) and in artificial waters in different botanical gardens (Palik, 1950). Pascher (1939) states that *Sphaeroplea* prefers limerich waters, and that it has never been found in dystrophic lakes.

Table II. Some chemical parameters and biological status for loc. 1-3 in 1993.

Loc. 1

Date	CND μScm^{-1}	Salt $g\ l^{-1}$	Biological status
05.07.93	4230	2,12	The pool is nearly dried out. Green threads of *Sphaeroplea annulina*. Both vegetative cells and cells with masses of ova and antheridia. Cells 30-35 µm broad, length of oogonia 375-600 µm, number of ova 21-80 per cell.
10.07.93	1600	0,80	Rainfall 10.07 filled all the pools with water. Some green filaments of *Sphaeroplea*.
11.07.93	1600	0,80	Some vegetative filaments. Most cells with verrucose, green to brown oospores.
20.07.93	1120	0,56	No algae. t = 19,2°C.
28.07.93	-	-	No algae.

Loc.2

Date	CND μScm^{-1}	Salt $g\ l^{-1}$	Biological status
05.07.93	-	-	The pool is dried out, except for two deeper parts where there was a wet cover of *Sphaeroplea*. The material consisted of vegetative cells and cells with masses of ova. Also plenty of antheridia which were filled with spermatozoids. Cells 20-25 µm broad, length of oogonia 350-900 µm, length of antheridia 300-550 µm, number of ova 30-38 per cell. A few slightly verrucose, green oospores.
10.07.93	-	-	Rainfall 10.07 filled all the pools with water.
11.07.93	380	0,19	Only vegetative filaments. In some cells differentiation had started.
20.07.93	460	0.23	Few threads only. Most are vegetative, but cells with verrucose, green oospores are also found. Some of this material was grown in a glass. These algae had already on 24.07 masses of ova and green,verrucose oospores. On 24.09 masses of red oospores. t = 20,7°C.
28.07.93	-	-	No algae found.

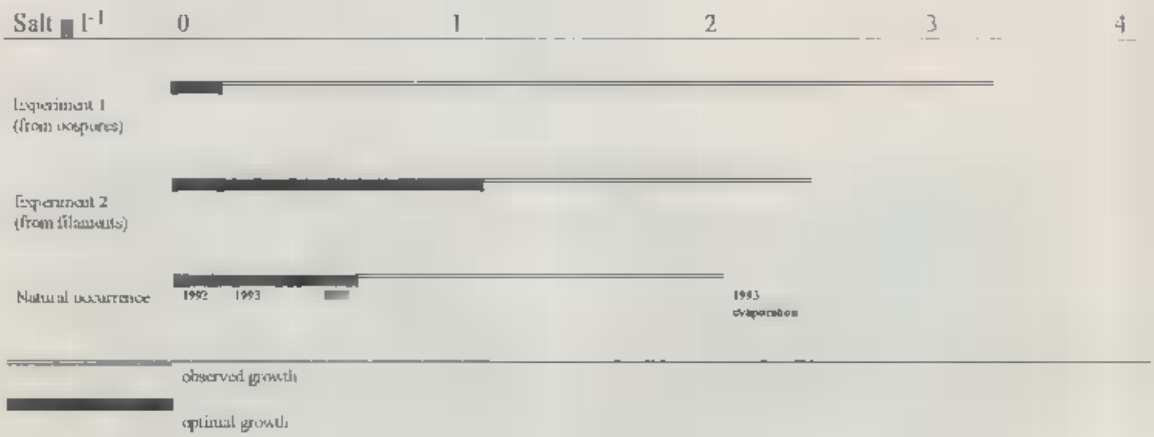
Loc.3

Date	CND $\mu S\ cm^{-1}$	Salt $g\ l^{-1}$	Biological status
05.07.93	-	-	The pool is dried out.
10.07.93	480	0,24	Rainfall 10.07 filled all the pools with water. No algae.
20.07.93	460	0,23	No visible algae. t = 20,9°C.
24.07.93	-	-	Green masses of filaments in the water. Only vegetative cells.
28.07.93	-	-	Many green filaments, much vegetative filaments. Many cells with ova and some with green oospores.

Most commonly the species has been reported from fresh water and slightly brackish water. This is supported by my own observations and experiments.

These results are given in table III.

Table III. Summary of the observed tolerance of *Sphaeroplea annulina* for salt (g l^{-1}) in two experiments and in natural occurrence. Most observed values for salt are relatively low, and indicate optimal growth in fresh water (< 0,5 ‰ l^{-1} salt) or slightly brackish water.



Germination of oospores was best in water with low salt content, but took place up to a salt content of around 3,6 g l^{-1}. Optimal growth of filaments was found to be up to 1,3 g l^{-1} of salt. In localities near the coast this property is probably necessary for survival, ■ the salt content here can vary considerably due to evaporation and spray from the sea. This is also observed in loc. no. 1 on 05.07.1993, where the salt content was 2,12 g l^{-1} due to evaporation , and where the alga grew very well.

In their investigation of the species in Spain, Cambra & Couté (1988) found *Sphaeroplea* in a rice-field with slightly brackish water with a conductivity of 2650 µS cm^{-1}. In my field observations I found the conductivity to vary between 180 - 4230 µS cm^{-1}.

The content of chloride indicates some influence from the nearby sea. Skuja (1927) found *Sphaeroplea annulina* near the coast in Lettland. In his paper he says that the water in one of the pools, where the species grew, tasted salty. Cambra & Couté (1988) found *Sphaeroplea* in a pool with slightly brackish water with a content of 730 mg Cl l^{-1}. My own measurement of chloride are incomplete. They are all made in fresh water (value below 100 mg Cl l^{-1}).

pH is normaly reported in the alcaline area, from 7,6 - 8,8 in Norway. Christensen (1954) gives 7,9 and Cambra & Couté (1988) gives 9,7 (high photo-synthetic rate).

Sphaeroplea annulina is a species well adapted to variations in the ionic content of water. The optimal growth of *Sphaeroplea annulina* occurs in fresh water to slightly brackish water. According to Økland (1983) the limit between these two types is 0,5 ‰ l^{-1} salt.

The species has also been found in eutrophic lakes in Denmark (Christensen, 1971) and in a high mountain lake in India, also eutrophic (Randhawa, 1958). Such localities are very different from the temporary pools, where the chances for drying out are considerable.

The specimens of *Sphaeroplea annulina* found in Norway fit in very well with the general description given for the species. Both width of filaments and diameter of oospores fit with Skuja (1927) and Gauthier-Lièvre (1941). Both Palik (1950) and Cambra & Couté (1988) give a bigger variation in the two characters.

Temperature is an important ecological factor affecting the growth of algae in general. In my field observations the temperature variation was 18,5° C - 24,0° C, and in the experiments c. 19° C - 25° C. In Budapest Palik (1950) measured 22 - 23° C in the watertanks there. Cambra & Couté (1988) give 29,2° C from their locality in Spain.

In the Norwegian localities *Sphaeroplea* is the dominating species (Fig. 5) only found together with *Oedogonium* sp. and *Scenedesmus* sp. Skuja (1927) reported *Sphaeroplea* mixed with *Ulothrix* sp., *Oedogonium lautumnarium* Wittr., together with a few species of *Scenedesmus*, *Ankistrodesmus falcatus* (Corda) Ralfs, *Nodularia spumigena* Mertens and *Pandorina morum* (Müller) Bory. In eutrophic lakes *Sphaeroplea* are found in vegetation mixed with phanerogames and other algae (Randhawa, 1958; Christensen, 1971; Cambra & Couté, 1988).

Sphaeroplea annulina complete its life-cycle in a relatively short time. In 1992 I found only vegetative filaments on 26.07 and only filaments with red oospores on 10. 08. In my culture experiments the total life-cycle was completed in about four weeks. Lowe (1924) states that "the algae which thrive here complete their life-cycle and produce resting spores in six to eight weeks at low temperatures".

In scientific papers *Sphaeroplea* is reported as a spring alga in Europe, where it normally can be found from April to June (Roth, 1806; Palik, 1950; Cambra & Couté, 1988). In Northern Europe most observations of the alga are from June-August (Skuja, 1927, Christensen, 1954; Langangen, 1992). In Norway red oospores were found in August both in 1992 and 1993.

It seems that *Sphaeroplea annulina* is well adapted to survive in small temporary pools, both inland but most commonly near the sea. The fast life-cycle and the massproduction of oospores are both adaptations to survive in such localities.

Acknowledgement - I am in debt to Dr. Oskar Garstein, Oslo who has read the manuscript and corrected the English language.

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